

Materials and methods

Xkr cDNAs

Primers used to prepare the *Xkr* cDNAs were as follows. The recognition site for the restriction enzyme (*Bam*H1, *Bgl*II, *Eco*R1 or *Mfe*1) is underlined for each primer.

m*Xkr1*, 5'-ATATGGATCCGAGATGAAATTCCCGGCCTCGGT-3' and

5'-ATATGAATTCAGCAGAGCACAGATCAACAG-3';

m*Xkr2*, 5'-ATATGGATCCCACAATGGACAGAGTTATGAAAT-3' and

5'-ATATGAATTCGACAATACTTGTGTTGT-3';

m*Xkr4*, 5'-ATATAGATCTTATCATGGCCGCTAAGTCAGACGG-3' and

5'-ATATGAATTCTAAAGTGGTTCATATTCCA-3';

m*Xkr5*, 5'-ATATGAATTCAAGATGCACGCCGGCTCCTGGG-3' and

5'-ATATGAATTCGATGAAAAAGCTCATCTCCT-3';

m*Xkr6*, 5'-ATATGGATCCAAGATGGCGCGAAATCCGATGG-3' and

5'-ATATGAATTCGAGTGAAGACTCATACTGCA-3';

m*Xkr7*, 5'-ATATGGATCCAACATGCCCGAAGTCGGATGG-3' and 5'-

ATATGAATTCTACTGTGGTCTCATACTCCA-3';

m*Xkr9*, 5'-ATATAGATCTTATAATGAAATATACCAAGTGTAA-3' and

5'-ATATGAATTCGTCCATTAGAAAATATCTTA-3';

h*XKR4*, 5'-AGATCTGAATTCATGCCGCTAAATCAGACGG-3' and

5'-CTCGAGCAATTGTAAGTGGTTCTGACTCCA-3';

h*XKR9*, 5'-GAATTCGGATCCCATGAAATATACTAACAGAA-3' and

5'-CTCGAGCAATTGTTCCATTAGGAAATATCTCA-3'.

Construction of m*Xkr* mutants

A point mutation was introduced in m*Xkr8* cDNA by recombinant PCR (1) using following mutagenesis primers.

A46E, 5'-TTATCTGTGGGAGGCGCTGGTA-3' and

5'-TACCAGGCTCCCACAGATAA-3';

S64L, 5'-GCTGCAGCTTCCCTCTGGCTCTG-3' and

5'-CAGAGCCAGAGGAAGAGCTGCAGC-3';

G94R, 5'-GCTGCAGCTCGCTACCTGTA-3' and

5'-TACAGGTAGCGGAGCTGCAGC-3';

L150E, 5'-CACACTGGTGGAGGCAATTGTA-3' and

5'-TACAATTGCCTCCACCAGTGTG-3';
G248T, 5'-CTGGCTTCAAACCACAAATT-3' and
5'-AAAATTGTGGTTGAAGCCAG-3';
D295K, 5'-CATCTTCAGTAAGAGTGTCTGCT-3' and
5'- AGCAGAACACTCTTACTGAAGATG-3';
V35A, 5'-GGGCCGTTGCCAGTACGTGC-3' and
5'-GCACGTACTGGCAACGGCCC-3';
E141R, 5'-GAGCTCCTGCGGGCGACGCCA-3' and
5'-TGGCGTCGCCGCAGGAAGCTC-3';
Q163T, 5'-GGAATACTACACGTGGTTGGC-3' and
5'-GCCAAACCACGTGTAGTATTCC-3';
S184V, 5'-TTACCATGGGTTCTGCGTACC-3' and
5'-GGTACGCAGAACCCGATGGTAA-3';
I216T, 5'-GGGCCAGAACCTGTGCCATC-3' and
5'-GATGGCACAGGTTCTGGGCC-3';
V305S, 5'-CACCTCCTGGTCGACACACGGC-3' and
5'-GCCGTGTGTCGACCAGGAGGTG-3';
T309F, 5'-GACACACGGCTCTGGCTGCC-3' and
5'-GGGCAGCCAGAACCGTGTGTC-3'.

The mutated mXkr8 cDNA was introduced into pMXs-puro c-Flag to produce Flag-tagged protein. Mutations in the caspase-recognition sites were introduced by recombinant PCR, using the following primers:

mXkr4 1DA, 5'-GCAGAGCGGGCTGGATGTGTAC-3' and
5'-GTACACATCCAGCCGCTCTGC-3';
mXkr9 2DA, 5'-CCACAACTTGCTGAAACTGCTGGAAAAGCA-3' and
5'-TGCTTTCCAGCAGTTCAGCAAGTTGTGG-3'.

The truncated forms of Xkr cDNA were generated by PCR, using the following reverse primers:

mXkr4 ΔC, 5'-GGATCCAATTGATCCCGCTCTGCAAATTCT-3';
mXkr8 ΔC, 5'-GAGATCTGAATTCCACGAGGTCAAGGGTCCC-3';
mXkr9 ΔC, 5'-GGATCCGAATTCCATCAGTTCATCAAGTTGTG-3'.

The mutated or truncated cDNAs were introduced into pMXs puro c-Flag or pMXs puro c-GFP to produce the Flag-tagged protein or GFP-fusion protein, respectively.

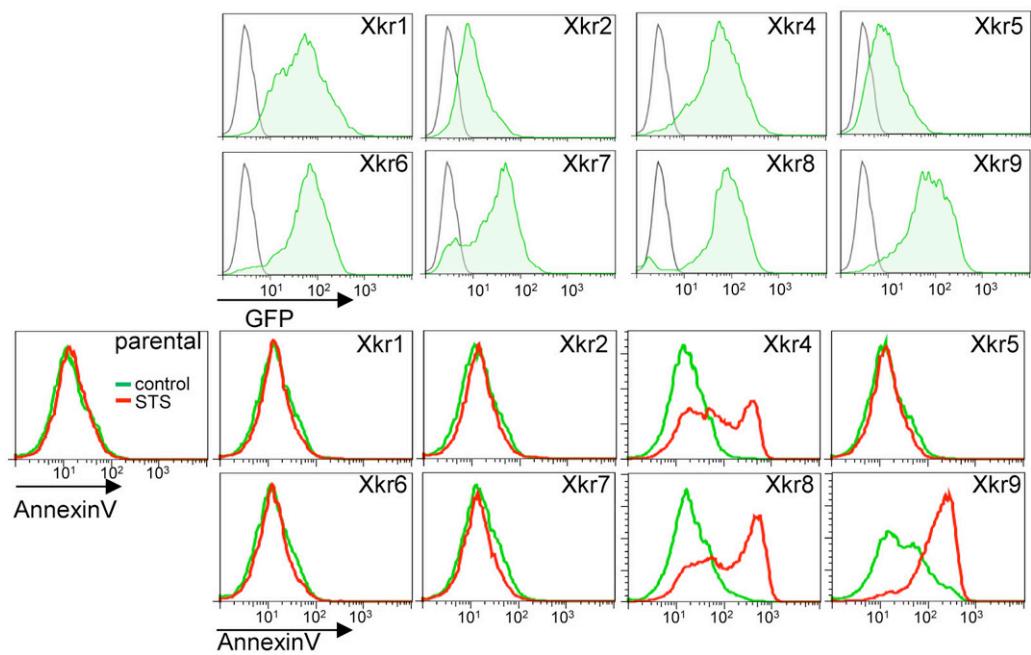
Real-time RT-PCR

Total RNA prepared from various mouse tissues using RNeasy Midi Kit (Qiagen, Venlo, Netherlands) was reverse-transcribed using Superscript III reverse-transcriptase (Invitrogen) or High Capacity RNA-to-cDNA™ Kit (Applied Biosystems, Foster City, CA). Specific cDNAs were amplified in a reaction mixture containing LightCycler®480 SYBR Green I Master (Roche Diagnostics, Basel, Switzerland), and the mRNA was quantified at the point at which the LightCycler System detected the upstroke of the exponential phase of PCR accumulation. pMXs-puro plasmid DNA carrying each *Xkr* cDNA was digested with *Nco*I and used as a reference. Primers for real-time RT-PCR were:

m*Xkr4*, 5'-GCCAGTGACCGTGATCAGAA-3' and 5'-TCCTTGTACTGCAGCCTTGG-3'; m*Xkr8*, 5'-GCGACGCCACAGCTCACACT-3' and 5'-CCCCAGCAGCAGCAGGTTCC-3';
m*Xkr9*, 5'-GGAAGGCTGCCGCAACTCA-3' and 5'-TGGGCCAGAGTCCTCGGAGAA-3';
m*Gapdh*, 5'-AGCAGGCATCTGAGGGCCA-3' and 5'-GAGAGCAATGCCAGCCCCGG-3';
h*XKR4*, 5'- TGCAGACGCATTGCCATT-3' and 5'-CAACACTGCGGTTGGAG-3';
h*XKR8*, 5'-AGGCCGGGCCATCATCCACT-3' and 5'-TGCGCCTGTTCTGAGGCAGC-3';
h*XKR9*, 5'-GCAGGCCAAGAAAGTCAGCATTGT-3' and 5'-TGGGCAGCCTCCAGGTAGGT-3';
h*β-actin*, 5'-GCATCCTCACCTGAAGTAC-3' and 5'-CTTAATGTCACGCACGATTTC-3'.

References

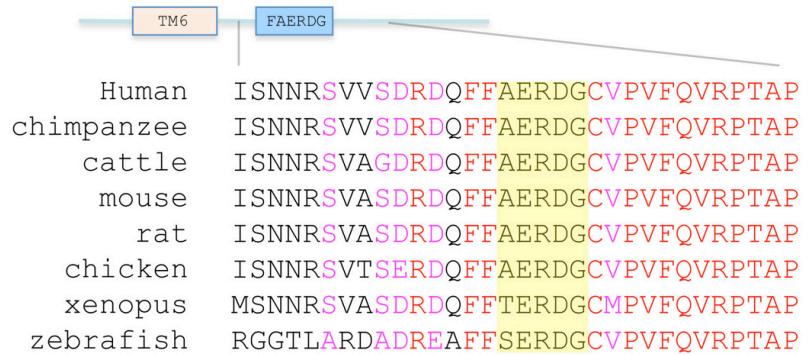
1. Higuchi, R. (1990) Recombinant PCR. in *PCR protocols: A guide to methods and applications*, Academic Press, San Diego. pp 177-188



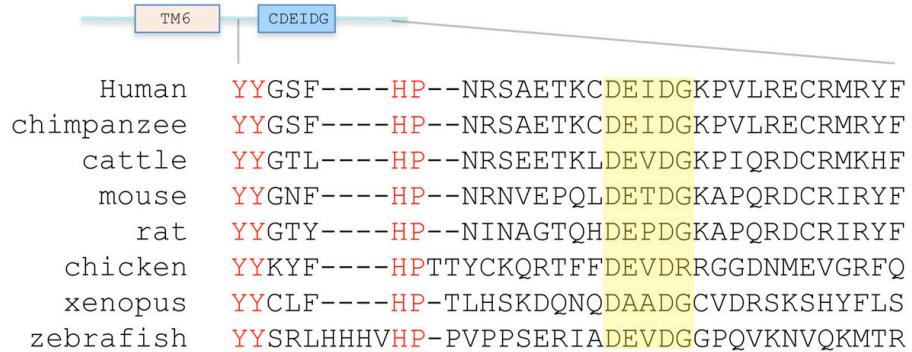
Supplemental Fig S1

Apoptotic PtdSer exposure in PLB-985 transformant cells expressing mXkr family members. PLB985 cells transformed with the indicated GFP-fused mXkr family members were treated with staurosporine (STS) and stained with Cy5-AnnexinV and PI. The GFP profile (upper panels) and Annexin V-staining profile in the PI-negative fraction (red, STS-treated; green, untreated) (lower panels) are shown.

Xkr4 C-terminal cytoplasmic region



Xkr9 C-terminal cytoplasmic region



Supplemental Fig S2

Amino acid sequence alignment of the caspase-recognition site in Xkr4 and Xkr9 of different species. About 30 amino acids around the caspase-cleavage site were aligned. Red: residues that are identical in all members. Pink: residues that are similar in all members. Yellow: putative caspase-recognition sites. TM6: sixth transmembrane region.